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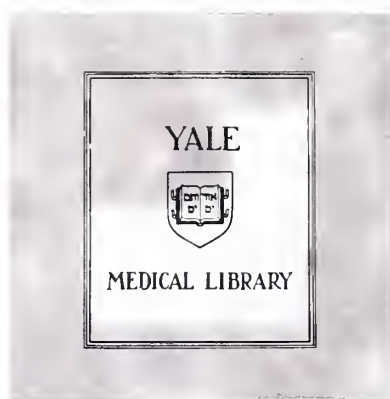


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CARBOHYDRATE INTOLERANCE
IN CHRONIC UREMIA

Bernard Lewin

1981





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CARBOHYDRATE INTOLERANCE
IN
CHRONIC UREMIA

BY
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A Thesis Submitted to
The Yale University School of Medicine
in Partial Fulfillment
of the Requirement for the Degree of
Doctor of Medicine.

1981

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Brief Introduction

The association of altered carbohydrate metabolism with renal failure has been described and investigated extensively since Neubauer first described it in 1910 (17,64,82). Nevertheless, the mechanisms underlying the glucose intolerance of uremia remain poorly defined. Elucidation of this mechanism might help to provide insight into the mechanism of insulin action in general as well as further define the factors contributing to other insulin resistant states such as obesity and diabetes mellitus (18,39,67).

The complexity of glucoregulatory mechanisms in man provides numerous sites at which inhibition or interference might result in the glucose intolerance commonly associated with uremia (24). Studies suggesting the possibility of a defect in insulin synthesis have not proven valid (17). However, an abnormality in the early phase of insulin secretion in uremia has been described (8,12,15,16,17). The role of other hormones that affect glucose metabolism has also been investigated. These evaluations of the role of glucagon (10, 15,44,45), growth hormone (24,48,57,84), parathyroid hormone (3,42,54), epinephrine (15), corticosteroids (15,17,64), and gut hormones (cholecystekinin, gastrin, secretin)(3) in the glucose intolerance of uremia have been extensive and on occasion, contradictory.

Because it is a central organ in the glucoregulatory pathways of the body, the role of the liver has been studied

from a number of different perspectives. Alterations in a) hepatic glucose uptake, b) hepatic glucose production, c) glycogenolysis, have all been extensively studied (9,15,16, 19,29,34,46,49,59,61,71,73).

Adding to this complexity are a number of metabolic toxins such as urea, creatinine, guanidosuccinic acid, methylguanidine, indoles, phenols, and aliphatic amines which accumulate in the uremic state and have been described to affect carbohydrate metabolism (5,8,9,14,17,30,49,64). Electrolyte abnormalities and inhibition of several different enzymes have also been implicated in this area (8,17,46,49,55,56,77,78). Many of these early studies were flawed by the need to perform in-vitro studies of complex in-vivo processes and by the inability of investigators to completely eliminate the complex neuro-endocrine response to experimental manipulation, hypoglycemia, etc.

By using the recently developed insulin clamp technique, the present study eliminates many of these problems. With this technique, a steady state plateau of hyperinsulinemia is created while the plasma glucose level is held constant at euglycemic levels by varying the rate of a separate glucose infusion. This avoids the neuro-endocrine response described above. Since the plasma glucose concentration is held constant at the basal level, the rate of glucose infusion must equal the rate of glucose uptake by all the tissues of the body and

provides a measure of tissue sensitivity to insulin.

In the present study, we have employed the euglycemic insulin clamp technique to examine tissue sensitivity to insulin in the unanesthetized uremic rats and normal controls so that some determination of the validity of this model in investigation of glucose intolerance in chronic uremia might be made.

Introduction

An understanding of the carbohydrate intolerance of chronic uremia requires familiarity with the factors that control glucose homeostasis and the points at which these mechanisms can be altered in the normal individual. This understanding becomes even more difficult in the uremic patient, in whom there are accumulations of metabolites not present in healthy subjects, and because of the marked derangements in hormone and electrolyte concentrations which also accompany uremia

I. Normal Carbohydrate Metabolism

Following the administration of an oral or intravenous glucose load, the plasma glucose concentration increases and the resultant hyperglycemia provides a stimulus to pancreatic beta cells to release insulin into the circulation. The released insulin facilitates the transport of glucose across the cell membrane, which in turn leads to a decrease in plasma glucose concentrations toward baseline levels. The majority of glucose absorbed after oral intake is retained in the splanchnic bed, with only 10-15% becoming available to peripheral tissues in the resting state (32). Under these conditions, then, splanchnic uptake remains the major determinant of the shape of the glucose tolerance curve (32).

While insulin is known primarily for its role in facilitating glucose transport into cells, this hormone is also involved in membrane transport of amino acids, anions, cations, and other hexoses (13). Muscle and fat are the tissues most dependent on the effects of insulin for transmembrane hexose transport. This occurs by a process of facilitated diffusion, implying that the concentration gradient across the membrane remains an important factor even in the presence of insulin (13).

Once the glucose is in the cell, it is phosphorylated and can no longer diffuse across the cell membrane. The glucose-6-phosphate may then be converted to uridine diphosphate glucose and be stored as glycogen or utilized in the synthesis of mucopolysaccharides. G-6-P might also be oxidized in the hexose monophosphate shunt to yield ribose sugars for RNA synthesis. More commonly, however, the G-6-P will be utilized in the Embden-Meyerhof glycolytic pathway.

In adipocytes, glucose transport into the cell is the rate limiting step of glucose metabolism. Once the glucose is in the cell, however, phosphorylation becomes the rate limiting step (11). Factors which inhibit other steps in this metabolic pathway to rates lower than either of these two steps will decrease the rate of carbohydrate metabolism.

II. Carbohydrate Metabolism in Uremia

There is general agreement that glucose metabolism is impaired in the majority of uremic patients. Such patients

demonstrate glucose intolerance not unlike that of adult onset diabetes mellitus. It becomes important to differentiate the glucose intolerance of uremia from that of diabetes mellitus primarily because diabetics have a significantly greater incidence of serious complications and mortality with hemodialysis or transplantation than non-diabetics (17).

The majority of uremic patients have fasting glucose levels in the normal to high range despite elevated fasting plasma insulin levels. This suggests the presence of insulin resistance. Following both oral and intravenous glucose loads, glucose tolerance has been shown to be abnormal (8,15,64). Approximately 50% of uremics manifest these signs (17,78). These include a delayed, diminished fall in plasma glucose after exogenous insulin administration in uremic patients (16,64). Uremics similarly manifest a delayed decrease in blood glucose after administration of oral hypoglycemic agents in the absence of either drug persistence or beta cell refractoriness (10).

The degree of insulin resistance demonstrated in abnormal glucose tolerance testing has not been shown to correlate with the severity of the azotemia (8,64). However, there is a positive correlation between fasting blood sugar levels and blood urea nitrogen in uremic patients (78).

III. Factors which might affect carbohydrate metabolism in uremia.

A- Insulin secretion

Investigation of the insulin secretory response of uremics

to a glucose load have been conflicting (17). Several investigators have reported a decreased plasma insulin response to both oral and intravenous glucose in uremics (44,45). However, an equal number of reports have demonstrated either a normal (48,72), or increased (49,56) plasma insulin response. These reports refer only to the early phase of insulin release which normally occurs from two to five minutes after elevation of plasma glucose levels and is felt to represent the release of packets of pre-formed insulin (25). The later, more gradual release that occurs throughout the period of hyperglycemia has been uniformly reported as increased in uremics after both oral and intravenous glucose loading (7,8,45,78). This later release is felt to represent the release of newly synthesized insulin.

DeFronzo (15) studied glucose intolerance in uremic patients using the glucose clamp technique. He explains that the differences between these above-mentioned reports are consistent with a spectrum of abnormalities ranging from patients with normal beta cell response to glucose to those with markedly inhibited insulin secretion. The former group would manifest normal fasting plasma insulin levels with an increased early and late response to glucose while the latter group would have low fasting plasma insulin levels and inadequate or diminished insulin secretion in response to glucose (15). In addition, tangential evidence that some uremics may have

inhibition of insulin secretion is provided by the finding that the response to intravenous tolbutamide is delayed and diminished in uremics (8,44,78), but that this inhibition is corrected by dialysis (44,56,78). Although insulin response may be inhibited in some uremics, this would not explain the elevated fasting insulin levels with normal plasma glucose levels found in almost all uremic patients.

B. Insulin metabolism

It has been demonstrated that chronic dialysis patients have a decreased rate of insulin degradation and an expanded body pool of insulin (33). This increased insulin pool is greater than that which would be produced by fluid retention due to renal failure (33). Another study attributes the hyperinsulinemia to a prolonged half life for insulin due to a decreased metabolic clearance rate and a prolongation of stimulation for pancreatic secretion secondary to the delayed, diminished decrease in plasma glucose seen in uremia (48).

Perhaps the most definitive studies of beta cell response are those using the glucose clamp technique. With this technique, the plasma glucose concentration is acutely raised by 125 mg. per 100 ml. and maintained at that level by a variable glucose infusion. Since all subjects have had their glucose levels raised by the same amount, the plasma insulin response provides a measure of beta cell response to hyperglycemia. Results of these studies have indicated that the early plasma

insulin response to hyperglycemia is similar in control subjects and uremics. The late response in uremics is almost uniformly increased secondary to increased secretion and decreased clearance. After dialysis, the plasma insulin response decreased by 32% due to an increase in the metabolic clearance rate of insulin and a reduction in insulin secretion. (15,16,17). Other studies have resulted in similar observations (7,8,12,48,50,78).

C. Alterations in hormone concentrations

In addition to insulin, the metabolism of several other hormones known to be important in glucose homeostasis have been reported as abnormal in patients with chronic renal failure. Plasma levels of glucagon have uniformly been reported to be increased (3,15,73). This is due at least in part to a marked decrease in the degradation of glucagon by the uremic kidney (73). Since glucagon is known to stimulate hepatic glucose production, it is possible that the fasting hyperglycemia observed in uremic patients as well as the excessive hyperglycemia following glucose administration is secondary to elevated basal hepatic glucose production and a failure of the liver to normally suppress gluconeogenesis and glycogenolysis following intravenous or oral glucose. Results of investigations in this area range from the reports by Cohen and Horowitz that patients with significant renal disease failed

to demonstrate an appropriate increase in plasma glucose in response to glucagon (10), to Hampers' report of normal response to glucagon in the uremic patients he tested (44,45). Others report either mild (8) or severe (15,73) hypersensitivity to the plasma glucose elevating effects of glucagon in uremics. There is somewhat more agreement on the question of glucagon levels in uremia. Nearly all authors reported normal levels of glucagon secretion despite elevated fasting levels which remained elevated throughout glucose tolerance testing (3,15,73). These elevations are attributed to a marked decrease in the metabolic clearance rate of glucagon in uremics relative to controls (73). Despite these observations, it is unlikely that this is the major cause of glucose intolerance in uremia. The major site of glucagon action is in the liver, where it stimulates both glycogenolysis and gluconeogenesis. It has been well documented that uremic patients demonstrate no increase in basal hepatic glucose production, and that such production is normally suppressed by insulin and glucose (15). In either case, dialysis results in decreased glucagon levels, some normalization of glucose tolerance, and a normal increase in plasma glucose levels in response to glucagon in uremic patients (27,73).

Hyperinsulinemia per se might contribute to some degree of glucose intolerance. Insulin is known to regulate its own

receptor number so that under conditions of hyperinsulinemia, a decrease in the number of insulin receptors takes place (19). This decrease might cause a relative resistance to insulin effect.

Elevated growth hormone levels might also contribute to carbohydrate intolerance. Elevated fasting growth hormone levels have been measured in many uremics (48). However, another study found that only 50% of uremic subjects demonstrated this finding, and that only 50% of these demonstrated an abnormal growth hormone response to glucose administration (24). This latter group manifested a paradoxical rise in growth hormone levels after glucose administration (24,48,72). Despite the frequent observation of this phenomenon, no correlation has been found between elevated growth hormone levels and the degree of carbohydrate intolerance in uremics. No change in fasting growth hormone levels is observed after dialysis. No correction of the paradoxical rise in growth hormone levels after glucose administration is seen after dialysis despite marked post dialysis improvements in carbohydrate metabolism (24,48,72). It is therefore unlikely that alterations in growth hormone levels or action can satisfactorily explain the glucose intolerance of uremia.

Two other hormones which have been specifically investigated for their role in glucose intolerance are parathyroid hormone and corticosteroids. In vitro studies of renal

tubules from starved rats show that elevated parathyroid hormone levels increased gluconeogenesis from lactate, pyruvate and glutamate (42). Bagdade observes that parathyroid hormone may have a proteolytic effect on muscle, thereby increasing the concentration of alanine, a gluconeogenic substrate (3). Despite these observations, there has been no correlation observed between elevated PTH levels and uremic glucose intolerance, suggesting that PTH is not the cause (54).

Plasma corticosteroid levels in uremics have been reported as normal (15) or slightly elevated (17) in uremics. Although elevated glucocorticoid levels could promote gluconeogenesis from pyruvate and glycerol, and steroids are known to cause cellular insulin resistance (3), there are no reports in the literature of corticosteroid elevations in uremia of sufficient magnitude to account for the observed effects, and no correlation between the degree of glucose intolerance and elevation of steroid levels (17).

D. Accumulation of toxic metabolites

A broad range of metabolic products which are normally cleared by the kidneys but which accumulate in renal failure have also been investigated as causes of glucose intolerance. The possibility that some of these might be important in this regard is suggested by the observed improvement in carbohydrate tolerance after dialysis (14,15,44,45). Some of the substances which have been looked at include urea, creatinine, guanidosuccinic acid, methylguanidine (15), indoles, phenols, and aliphatic amines (3,10). Further evidence

for the role of a dialyzable metabolic product contributing to uremic glucose intolerance is the finding that a selective low protein diet can correct the intravenous glucose tolerance test in uremics who had previously demonstrated glucose intolerance (75).

The first two such metabolites studied were urea and creatinine, probably because of their premier position as metabolites commonly used to assess renal function. Several studies have reported some degree of correlation between the severity of carbohydrate intolerance and the degree of elevation of urea and creatinine concentrations (44,49,78). In vitro studies examining the effect of urea on glucose metabolism have produced conflicting results. In one study, incubation of rat hemidiaphragms with plasma from uremic rats failed to alter glucose metabolism (13). Another similar study revealed that rat diaphragm incubated with uremic serum utilized less glucose than rat diaphragm incubated in serum from normal subjects, thereby demonstrating insulin resistance (30). In vivo studies have also been conflicting. A single patient with normal glucose tolerance developed carbohydrate intolerance after urea was added to his dialysate (49). This is in contrast to Hampers' report of correction of glucose intolerance in uremic subjects dialyzed against a urea bath (44). Abnormal glucose tolerance was shown to develop in three male volunteers after three days of ingesting sixty grams per day of urea (64). The conflicting

reports about the direct effects of creatinine and urea on carbohydrate metabolism suggest that these are not principally responsible for the observed glucose intolerance (5). While the concentrations of these two metabolites are the more commonly used indicators of renal disease, other metabolites increase in concentration to parallel the degree of renal failure as well. It may be one of these other metabolites not commonly measured which accounts for the correlation between urea and creatinine levels and glucose intolerance (44, 78). Exactly which of the multitude of toxic metabolites this might be is unclear. Guanidosuccinic acid has been considered on the basis of its theoretical ability to inhibit phosphorylation of ADP to ATP (10). However, no such inhibition has been demonstrated in rats either in vivo or in vitro (9). In summary, there is little direct evidence to support the concept that any of the above mentioned metabolites is solely responsible for the carbohydrate intolerance of uremia.

E. Alterations in electrolyte concentrations

Alterations in electrolyte concentrations are common in uremic patients and could conceivably contribute to the impairment of carbohydrate metabolism.

Potassium levels are normally increased in chronic renal failure. After dialysis they are either unchanged from pre-dialysis levels (45) or slightly decreased (17). In addition,

most authors report improved glucose tolerance after dialysis. Only markedly decreased potassium levels have been shown to have detrimental effects on glucose tolerance (38), most probably because potassium has a direct stimulatory effect on pancreatic insulin secretion in vitro (41). While one study reported improved glucose tolerance after potassium replacement on patients with low levels of total exchangeable body potassium (59), another similar study found no such improvement (49). The fact that most uremic patients are hyperkalemic and the lack of correlation between either serum potassium levels or total exchangeable potassium levels makes it highly improbable that alterations in potassium levels plays a significant role in the carbohydrate intolerance of uremia (8,17,49,77,78).

Calcium is another ion with the potential for altering glucose metabolism when its concentration varies from the normal range, which does occur in uremia. An elevated serum calcium level allows normal pancreatic insulin secretion but results in decreased peripheral sensitivity to the released hormone (2). Low serum calcium levels cause a marked reduction in pancreatic insulin secretion without altering tissue sensitivity to insulin. Either of these situations might result in a decrease in glucose utilization and an abnormal glucose tolerance test. Evidence suggests that uremic patients may be even more sensitive than normals to these effects. While calcium infusion in hypocalcemic uremic patients normalized

their glucose tolerance tests, equally hypocalcemic control subjects had no evidence of glucose intolerance at all (55). The calcium infusion did not measurably increase basal plasma insulin concentration in the uremics, however, so the mechanism which might account for the improvement remains uncertain (55).

There is no evidence to support the idea that other ions might cause uremic glucose intolerance. Patients with chronic renal failure usually have elevated phosphate levels. Only markedly decreased phosphate levels have been shown to result in decreased glucose tolerance. While diet-induced hypophosphatemia decreased glucose clearance by 30% in dogs, their response to exogenous insulin was then more marked than that of controls (46). This is in marked contrast to the insulin resistance which accompanies uremic carbohydrate intolerance. Alterations in magnesium levels have similarly not been found to correlate with glucose intolerance, and no change in serum magnesium levels is found after dialysis despite improved glucose tolerance (45,72).

While acidosis has been found to result in abnormal responses to intravenous glucose and to exogenous insulin, the degree of acidosis commonly found in renal failure is of itself not sufficient to produce the observed effects (18).

F. Decreased insulin clearance

Certain changes which accompany renal failure may of themselves contribute to the observed hyperinsulinemia. As

discussed above, hyperinsulinemia causes a decrease in the number of insulin receptors and thereby creates a relative insulin resistance. The normal kidney cells of the proximal tubule actively remove insulin from the tubular lumen and from the peritubular cells. Only resorption from the lumen depends on oxidative metabolism. When the glomerular filtration rate falls in renal failure, the net rate of insulin removal from the renal plasma decreases despite a compensatory increase in peritubular absorption (65). The renal tubular cells then degrade the insulin, thereby reducing circulating insulin levels (27). Thus, the half life of insulin in uremic or anephric patients is markedly prolonged. The contribution of this increased half life toward uremic hyperinsulinemia can be calculated by measuring the difference in insulin concentration between the renal artery and vein in both normals and uremics. When this was done (70), a 30-40% decrease in insulin concentration was measured across the kidney. Accounting for plasma flow, 10-15% of the daily basal insulin output is shown to be degraded by the kidney. This can be reduced to as little as 2% of normal in severe renal failure (70). Decreased renal clearance of insulin alone does not explain the improved glucose tolerance and increased metabolic clearance rate of insulin after dialysis. Suppression of insulin degradation at another site such as the liver by a dialyzable toxin must

also be considered.

There is also evidence to suggest that other peptides are handled in the same way as insulin in renal disease. This might account for increases in levels of circulating ACTH, growth hormone, and glucagon (70). The fractional extraction of glucagon across intact rat and dog kidneys, for example, is normally 40%. This reduces to near zero in severe renal failure (73). This results in decreased clearance and a resultant prolonged half-life for these peptides. It is, in fact, this mechanism which is considered responsible for the decreased insulin requirements of diabetic patients who subsequently develop renal failure.

IV. Mechanisms of Observed Carbohydrate Intolerance

Having described the possible roles of all those factors which might contribute to abnormal glucose tolerance in uremia, it remains to discuss the sites of these effects. Two distinct possibilities emerge if we assume that, as the studies show, insulin secretion in uremics is adequate. Either 1) there is a problem with hepatic glycogen storage and release and/or a failure of the liver to suppress hepatic glucose production normally in response to insulin, or 2) there is peripheral resistance to the effects of insulin.

A. 1. Hepatic glycogen

If the liver were not taking up an appropriate amount of

glucose from an oral or intravenous load, one might expect that hepatic glycogen stores would be decreased. Several studies have demonstrated that such is not the case. First, as noted above, the plasma glucose response to glucagon is either normal or increased in uremia. Since the major source of this glucose is hepatic glycogenolysis, glycogen stores must be adequate. Secondly, direct measurement of glycogen in the livers of chronically uremic rats have found no differences between uremics and controls (6,29). Another mechanism for raising plasma glucose levels is gluconeogenesis. Studies by Rubenfeld and Garber suggest that basal hepatic glucose production in uremia is significantly elevated. One attempt to quantify this difference placed it at 38% above control levels(69). This study has been questioned because of their use of a glucose labelled in the #2 position, which has been found to overestimate glucose turnover in other studies due to "futile cycles" (1). Nevertheless, another study using different tracers has reported an increased level of gluconeogenesis in the uremic liver (69). Theories about the cause of this increase include the idea that the increased protein catabolism found in uremia provides elevated levels of amino acids which are gluconeogenic substrates (34). Normal inhibition of protein catabolism by insulin is felt not to occur (36). Certainly, the enzymes required for gluconeogenesis are present at higher levels than those of controls.

SGPT and SGOT levels are elevated in the livers of uremic rats (34). Phosphoenol pyruvate carboxykinase activity increases more rapidly in response to fasting in uremic rats than in controls (4), and this is accompanied by a greater increase in serum alanine, free fatty acids and glycerol in uremics than in controls. All of these changes suggest greater propensity toward gluconeogenesis in uremic subjects (4).

A.2. Suppression of hepatic glucose production.

Despite this biochemical predisposition toward gluconeogenesis in the basal or fasting state, there is nearly uniform agreement that hepatic glucose production is normally suppressed by physiologic levels of insulin (15,16,25,60). In humans, insulin secretion was found to decrease hepatic glucose production from basal levels by approximately 87% in both uremic and control subjects tested by insulin clamp (15,16,19). Another study reports similar suppression and notes no difference in the suppressive effects of insulin on hepatic glucose production either before or after dialysis (25). Similar studies in chronically uremic rats showed an unquantified but steady decline in hepatic glucose production after insulin administration (71), while another study in acutely uremic rats showed no difference in suppression of hepatic glucose production between uremics and controls (60). It is clear from these studies that despite the presence

of increased gluconeogenic capability in uremia, hepatic glucose production is normally suppressed by insulin. This makes it improbable that elevated hepatic glucose production plays a major role in uremic carbohydrate intolerance.

B. Insulin resistance

Finally, there is the possibility that peripheral resistance to the effects of insulin is a major factor in the uremics' altered carbohydrate metabolism. Several sources suggest that this is the case. The observation of a decreased response of muscle and fat to insulin in uremia is a nearly constant one. Using the isolated, perfused hindlimb preparation, Mondon, Dolkas, and Reaven (60) have shown that the ability of insulin to increase glucose uptake in uremic rat tissues was markedly decreased compared to controls. Fore-arm perfusion studies in humans by Westervelt (82,83) revealed that perfusion with insulin resulted in an increase in glucose uptake in uremics only 25% as great as in controls. This same study showed that there was a marked decrease in phosphate uptake in uremics, but that lactate production per unit of glucose taken up was equal in both groups. This implies an intact Embden-Meyerhof pathway but inhibition of either glucose transport into the cell or intracellular phosphorylation. The other phenomena observed in uremics, namely elevated fasting insulin levels with normal or slightly elevated fast-

ing blood sugar and delayed, diminished response to exogenous insulin are both most consistent with peripheral insulin resistance (17). Little is known about how this occurs, other than the decreased phosphate uptake mentioned above. There are numerous sites in the glucose metabolic pathways at which phosphorylation is a key step essential to normal utilization. Rat diaphragm incubated in uremic serum had a higher ratio of free glucose within the cell relative to concentrations in the medium than diaphragm incubated in normal serum(30). This suggests inhibition of glucose phosphorylation intracellularly. The same study revealed that despite utilizing less glucose than rat diaphragm incubated in normal serum, concentrations of glucose-6-phosphate and fructose-6-phosphate increased while the concentration of fructose-1,6-diphosphate fell. This suggests that the inhibited enzyme is phosphofructokinase (30). Luke, et al, in another study (58), reported that despite abnormal glucose tolerance tests, uremic patients had fructose tolerance tests nearly identical to those of controls. Since fructose metabolism occurs primarily in the liver and its transport is not insulin dependent, the authors concluded that there was no generalized abnormality of carbohydrate metabolism in the liver, and that the Embden-Meyerhof glycolytic pathway functioned normally below the level of fructose-1,6-diphosphate.

The fact that these studies are in such close agreement despite being conducted by different investigators in different tissues (muscle (30) and liver (58)) strongly suggests that inhibition of phosphofructokinase by uremic serum is a major contributor to the abnormal glucose metabolism observed in uremia.

Despite these extensive investigations and resultant evidence for the roles of numerous metabolic alterations which accompany renal failure and might cause insulin resistance, no single mechanism has been proven to account for the full extent of the alteration in glucose metabolism. It is highly probable that the total phenomenon is a summation of a spectrum of lesser alterations which exist in different combinations and degrees in individual patients.

Methods

Male Sprague-Dawley rats were received and housed in a constant temperature room with a twelve hour day-night cycle. Insulin clamp studies were performed on animals which had been fasted overnight but allowed free access to water.

Experimental (uremic group) animals were received one month prior to study as weanling males. They were housed under the conditions described above for five days prior to any manipulation. On the fifth day, a remnant nephrectomy procedure was performed on the right kidney. Animals were anesthetized with intra-peritoneal Nembutal (0.08 mg. per 100 grams body weight), then shaved over the right flank. A small, vertical skin incision was made and carried through the muscle layers to expose the right kidney. This was gently lifted from position and placed in a specially made retractor just outside the incision with all structures intact. The renal capsule was then incised near the renal pelvis to expose the structures at the uretero-vascular pole. Under a dissecting microscope, all branches of the renal artery were identified. Leaving only the single, most cephalad branch intact to perfuse the upper pole, all other branches were ligated with 4-0 silk suture material. Visual inspection of the kidney revealed that only 10-15% of the renal tissue remained perfused after this procedure. The kidney was then

gently re-inserted into its original retroperitoneal position and the wound closed in a single layer with stainless steel clips. The animals recovered quickly, were housed in individual cages and fed a prepared commercial rat diet from that point until the night before the clamp study.

One week after the 85% right nephrectomy procedure, the left kidney was entirely removed through a flank incision as described above. The uremic group consisted of ten animals.

Ten rats served as a control group. These were treated in an identical fashion to the uremic rats except that the nephrectomy was not performed.

Two weeks after the second nephrectomy procedure and four days prior to study, chronic indwelling catheters (PE-50 polyethylene tubing) were placed into the jugular vein and carotid artery of both the uremic and control rats. The venous catheter provided access for infusion into the rat while the carotid catheter was for the non-traumatic removal of blood for glucose and insulin determinations.

Catheter placement

For the catheter placement, animals were once again anesthetized using intr-peritoneal injection of Nembutal at 0.08 mg. per 100 grams body weight, then shaved at the neck

and at the nape of the neck. A vertical midline incision was made in the skin from a point midway between the mandibular angles to the sternal notch. The skin was reflected on the right side and blunt dissection carried down to expose the jugular vein. The vein was then ligated approximately 5 mm. above its convergence with the cephalic vein and a small nick made in the vessel just below the ligature. A PE-50 polyethylene tube with a prepared tapered tip was inserted through the nick and threaded through the superior vena cava into the right atrium. The catheter was tied in place and 0.2 cc. of a solution of saline and 100 units per ml. of heparin was flushed through the tubing to prevent clotting within the catheter lumen. The external end of the tubing was then plugged.

The right carotid artery was then exposed by incising the aponeurosis and reflecting the sternocleidomastoid and the strap muscles. The artery was then carefully dissected off the accompanying vagus nerve and elevated. A ligature of 4-0 silk was then placed on the artery as far cephalad as possible. A small serrafine clamp was then placed across the vessel as proximally as was practicable in each animal. A small nick was made in the artery proximal to the ligature and a catheter identical to the one placed in the jugular vein was inserted and held securely in place while the serrafine clamp was removed. The catheter was then advanced carefully

through the brachiocephalic artery into the aorta. This arterial catheter was then flushed with heparinized saline and capped after being secured with 4-0 silk ties.

Both arterial and venous catheters were then routed subcutaneously to exit via a skin incision at the back of the animals head. All animals recovered rapidly from this procedure, moving, eating, and drinking within three hours. All catheters were flushed daily with 0.2 cc. of the heparinized saline solution to maintain patency.

All of the nephrectomized animals developed clinical signs of the uremic syndrome within four days. These included pallor of the eyes, ears, and tail, dryness and coarseness of hair, and irritability with occasional fine twitches (4). Their rate of weight gain was also decreased.

Four days after catheter placement, euglycemic hyperinsulin clamp studies were performed to quantify tissue sensitivity to insulin in both groups.

Euglycemic insulin clamp

Animals were fasted from 6 P.M. of the night before study until after completion. During the study, animals were placed in a small plexiglass cage which allowed them enough room to rest comfortably but not move excessively. Cages were clear, well ventilated, and allowed access to the jugular and venous catheters through a slot in the top of the cage.

An insulin infusion was prepared at a concentration of 40 thousand micro-units per ml. using crystalline porcine insulin (U-40, Lilly Co., Indianapolis, Indiana). This infusate was made up in normal saline containing 300 mg. serum albumin per 100 ml. to minimize adherence of insulin to glassware or plastic tubing. All lines were flushed with an insulin free saline-albumin solution prior to their use in the insulin clamp study. Over the first eight minutes of the study an empirically determined priming dose of insulin designed to raise the plasma insulin concentration by approximately 100 micro-units per ml. was administered. This was followed by continuous infusion of insulin at a rate of 4.4 micro-units per gram of body weight per minute for a total of 120 minutes. The insulin perfusion was administered via Harvard perfusion pump.

During the two hour period of insulin infusion, the plasma glucose concentration was maintained in the euglycemic range with a variable infusion of 5% dextrose in water. During the first four minutes of the study, no glucose was infused. From four to ten minutes, glucose infusion was begun at 10 micrograms per gram of body weight per minute. From 10 minutes until the end of the study, the arterial plasma glucose concentration was determined at 5 to 10 minute intervals. Alterations were made in the glucose infusion rate to maintain the plasma glucose concentration constant at the desired level. Insulin levels were drawn at 30 minute intervals.

Table 1
The Prime-Continuous Insulin Infusion
Employed During the Euglycemic Insulin Clamp.

Time (min.)	Insulin infusion rate micro U./gram/min.
0-2	8.4
2-8	6.0
8-120	4.4

Computations-Glucose metabolism

During the first twenty minutes of insulin/glucose infusion, the plasma glucose concentration dropped by 15-20 mg. per deciliter. Thereafter, it was maintained within a narrow range of the twenty minute value. Under these steady state conditions of euglycemia, the rate of glucose infusion must be equal to the amount of glucose taken up and metabolized by all the tissues of the body. Therefore, the glucose infusion rate provides a measure of insulin-stimulated glucose metabolism. This assumes that there is no source of endogenous glucose production during the study, i.e. that hepatic glucose production is completely suppressed. To the extent that this is incorrect, the total rate of glucose metabolism will be underestimated. Although this study does not quantitate hepatic glucose production, previous studies in man and in rats have shown that hepatic glucose production is decreased by 90-95% when the plasma insulin concentration is raised by 100 micro-units per ml. More importantly, there is no significant difference in this suppressive effect between normal and uremic subjects (15,16,19,25, 60,71). Preliminary studies to develop an adequate method for determining hepatic glucose production in rats using a radioactive tracer technique have been performed. However, until this technique is further perfected, rates of glucose metabolism measured as glucose infusion rates may underestimate

the actual rate of glucose metabolism.

Studies by DeFronzo and Ferrannini (21) have shown that elevation of the plasma glucose concentration will increase the rate of glucose metabolism in direct proportion to the increase in the plasma glucose level. Since the steady state plasma glucose concentration during the clamp differed slightly between the uremic and control groups, the results are also expressed in terms of glucose clearance. (mean rate of glucose metabolism/steady state plasma glucose concentration).

The mean of the plasma insulin concentrations at 30, 60, 90, and 120 minutes was used to determine the steady state plasma insulin level.

Analytic methods

Plasma glucose determinations were performed on 50 micro liter aliquots of plasma by the glucose oxidase method (Yellow Springs Instruments, Model 23A glucose analyzer, Yellow Springs, Ohio). Plasma insulin determinations were performed by a radioimmunoassay technique.

Results

Fasting plasma insulin levels prior to the initiation of insulin infusion were 31 ± 3 $\mu\text{U/ml}$ in the uremic rats, but only 21 ± 4 $\mu\text{U/ml}$ in the control animals ($p < .05$). Steady state plasma insulin expressed as the average of half hourly measurements begun 30 minutes after the prime-continuous insulin infusion was begun (Table 1) were 141 ± 8 $\mu\text{U/ml}$ in the uremic group, but 149 ± 16 $\mu\text{U/ml}$ in controls, (table 2)

After a 16-18 hour fast, baseline plasma glucose concentrations were higher in uremics (146 ± 5 mg/dl) than in the controls (120 ± 5 mg/dl) ($p < .001$). Steady state plasma glucose concentrations were 120 ± 4 mg/dl in the uremic rats and 104 ± 2 mg/dl in control animals ($p < .01$). The mean decrease in plasma glucose over the course of the study was 25.44 mg/dl in the uremic group and 15.51 mg/dl in the control group ($p < .05$). (Table 2).

Glucose metabolism over the course of the 120 minute study averaged 12.17 ± 0.70 micrograms/gram/minute in uremics and 16.08 ± 0.89 micrograms/gram/minute in controls ($p < .001$) (Tables 3 and 4). The rate of glucose metabolism rose very rapidly during the first 30 minutes of glucose and insulin infusion, then levelled off to nearly constant values (Tables 3 and 4)

Glucose clearance values were calculated to eliminate

error introduced by differences in steady state plasma glucose concentrations between the two groups (Table 6). Studies by DeFronzo and Ferrannini (21) have shown that for a given plasma insulin concentration, glucose clearance is constant despite changes in plasma glucose concentration within the physiologic range.

Glucose clearance averaged 10.28 ± 0.78 ml/kg·min in the uremic rats, but was significantly higher at 15.55 ± 1.1 ml/kg·min in control rats under similar conditions of hyperinsulinemia. ($p < .001$) (Table 6 and Figure 1). In parallel with glucose metabolism, glucose clearance increased greatly from the first twenty minute time period to the second, then tended to level off to a much slower rate of increase for the remainder of the 120 minute study. (Figure 1).

Table 2
 Characteristics of Uremic Animals and Controls

	Body Weight (grams) (1)	Fasting Plasma Glucose (mg./dl) (2)	Steady State Plasma Glucose ** (3)	Fasting Plasma Insulin (μ U/ml.) (4)	Steady State Plasma Insulin * (5)
Uremics (n=10)	155 ± 8	146 ± 5	120 ± 4	31.3 ± 3	141 ± 8
Controls (n=10)	141 ± 4	120 ± 5	104. ± 2	21.4 ± 4	149 ± 16

Values expressed as mean $\pm$ SE.

* Mean insulin concentration from 30-120 minutes.

** Mean glucose concentration from 0-120 minutes.

(1) p less than .05

(2) p less than .001

(3) p less than .01

(4) p less than .05

(5) p less than .3 (not significant)

Table 3

Summary of Mean Rates of Glucose Metabolism in UREMIC animals
During Euglycemic Insulin Clamp

Subject	Glucose Metabolism Rate - micrograms/gram/minute				
	Time Intervals (minutes)				
	0-20	20-40	40-60	60-120	20-120
1	8.30	10.50	12.00	12.00	11.70
2	9.80	13.50	14.50	10.83	12.10
3	9.55	13.50	15.00	16.91	15.85
4	8.50	12.25	11.00	10.50	10.95
5	8.00	11.50	14.00	18.50	16.20
6	9.50	15.00	17.00	19.33	18.00
7	7.50	9.50	10.50	11.00	10.60
8	8.50	9.00	11.00	10.00	10.00
9	9.00	14.00	12.50	11.33	12.10
10	7.00	9.00	13.00	13.33	12.40
Mean	8.57	11.77	13.05	13.73	12.99
±SE	0.29	0.69	0.65	1.12	0.85
					12.17 (1)
					0.70

(1) p less than .001 with respect to control group.

Table 4

Summary of Mean Rates of Glucose Metabolism in CONTROL animals
During Euglycemic Insulin Clamp.

Glucose Metabolism Rate - micrograms/gram/minute

Time Intervals (minutes)

Subject	0-20	20-40	40-60	60-120	20-120	0-120
1	13.45	18.00	19.40	26.40	23.32	21.68
2	13.85	18.75	21.00	21.66	20.95	19.77
3	13.45	17.75	16.25	16.00	16.40	15.91
4	11.60	16.50	17.00	19.00	18.10	17.02
5	11.60	15.00	14.25	15.25	15.00	14.43
6	10.80	17.50	17.00	18.33	17.90	16.71
7	11.55	15.75	15.00	15.50	15.45	14.80
8	8.55	11.50	15.35	17.17	15.65	14.46
9	10.60	15.75	13.25	12.00	13.00	12.60
10	8.80	12.00	14.00	15.17	14.30	13.38
Mean	11.43	15.85	16.24	17.65	17.00	16.08 (1)
±SE	0.58	0.77	0.77	1.27	1.00	0.89

(1) p less than .001 with respect to uremic group.



Table 5
Mean Glucose Values During
Euglycemic Clamp 0-120 minutes

mg./dl.

Subject	UREMIC mean $\pm$ SE		Control mean $\pm$ SE	
1	111	$\pm$ 4	97	$\pm$ 6
2	110	$\pm$ 3	95	$\pm$ 5
3	99	$\pm$ 4	107	$\pm$ 5
4	105	$\pm$ 3	100	$\pm$ 3
5	128	$\pm$ 5	112	$\pm$ 3
6	123	$\pm$ 5	104	$\pm$ 3
7	138	$\pm$ 6	108	$\pm$ 3
8	125	$\pm$ 3	106	$\pm$ 2
9	137	$\pm$ 3	110	$\pm$ 2
10	130	$\pm$ 4	105	$\pm$ 3
Mean	121		104	
SE	$\pm$ 4		$\pm$ 2	

All values expressed as mean $\pm$ S.E.



Table 6
Mean Rate of Glucose Clearance
from 0-120 minutes

UREMICS

subject	mean glucose metabolism rate (mg/kg/min)	mean glucose concentration (plasma) (mg/ml)	mean glucose clearance rate (ml/kg·min)
1	11.13	1.10	10.12
2	11.71	1.10	10.65
3	14.80	0.98	15.10
4	10.54	1.04	10.13
5	14.00	1.28	10.94
6	16.58	1.23	13.48
7	10.08	1.38	7.30
8	9.75	1.25	7.80
9	11.58	1.37	8.45
10	11.50	1.30	8.85
Mean	12.17	1.20	10.28
±SE	0.70	.044	1

CONTROLS

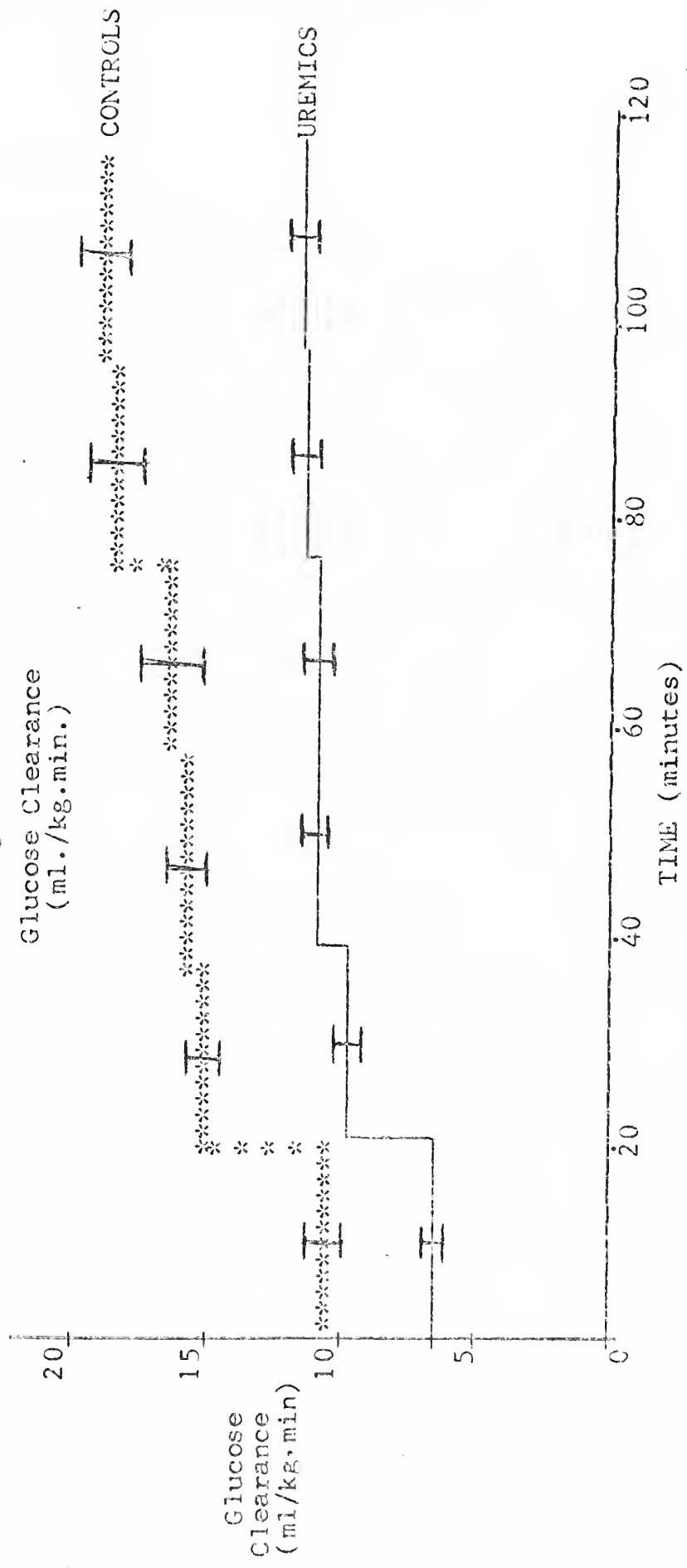
1	21.68	0.97	22.35
2	19.77	0.95	20.81
3	15.91	1.07	14.87
4	17.02	1.00	17.02
5	14.43	1.12	12.88
6	16.71	1.04	16.07
7	14.80	1.08	13.70
8	14.46	1.06	13.64
9	12.60	1.10	11.45
10	13.38	1.05	12.74
Mean	16.08	1.04	15.55
±SE	0.89	.018	1

$p < .001$

$p < .01$

$p < .001$

Figure 1



Values represent mean $\pm$ SE for each twenty minute interval

Discussion

This present study has reviewed the literature pertaining to carbohydrate intolerance of uremia and considered the mechanisms through which this phenomenon might occur and the sites at which these various factors might exert their influence to produce the observed effects. In addition, it has examined the validity of the uremic rat model for use in the study of this phenomenon by use of the euglycemic insulin clamp technique. (15,19,23) This technique offers many advantages over previous methods of investigation of glucose metabolism. It provides a means for study of the live, unanesthetized rat. It also allows the study of sensitivity to exogenous insulin over a prolonged time period during which equilibrium may be achieved. The complex neuro-endocrine response to hypoglycemia which plagued previous such studies is avoided, since the subject never becomes hypoglycemic. Previous studies such as the insulin tolerance test (8,64) and quadruple infusion techniques (68) relied upon pharmacologic manipulation to block these responses. Whether this block was complete or free of other effects is not known.

The current study supports the presence of fasting hyperinsulinemia which has been reported in previous studies (8,64). It is also consistent with reports of elevated fasting blood sugar levels reported by several authors in the uremic subjects

they studied (8,17,64,78).

Previous reports of a diminished response to insulin in uremic subjects relative to controls were supported by the results of this study (8,16,19,44,78). Examination of the graph of mean glucose clearance rate for each twenty minute time period reveals that the lines for each group are nearly parallel, but that the glucose clearance rate for the uremic group is significantly lower at all points ($p < .001$).

The mean rate of glucose metabolism for each of the two groups demonstrates a similar relationship, however, recent studies by DeFronzo and Ferrannini (21) have shown that for a given level of plasma insulin the glucose clearance rate remains constant despite differences in plasma glucose concentrations within the physiological range. Glucose clearance rates would therefore seem to be a better indicator of glucose metabolism than glucose metabolism rates alone. This is undoubtedly due to the fact that insulin stimulated glucose transport occurs by a mechanism of facilitated diffusion (13). As a result, differences in plasma glucose concentration effectively alter the concentration gradient across the cell membrane of free glucose. This results in a steeper gradient and an increase in glucose uptake per unit time. The use of glucose clearance rates cancels out the effect of such differences.

Having demonstrated a diminished response to exogenous insulin in uremic rats (i.e.-insulin resistance), it remains to discover the site of such resistance. Insulin exerts its glucose transport effect in peripheral tissues such as muscle and fat. If these tissues were resistant to insulin effect then the present observation of decreased glucose utilization would be the expected result. However, the possibility also exists that the observed phenomena are not the result of decreased transport, but of a failure of hepatic glucose production to be normally suppressed by hyperinsulinemia (15). Some attempts were made to address this question through the development of a tritiated glucose tracer technique for measuring the rate of appearance (R_a) of glucose during the euglycemic insulin clamp. Since the animals were fasted prior to study there would be no glucose appearance through absorption from the gastrointestinal tract. Any difference between the R_a for glucose and the known rate of infusion could then be attributed to hepatic glucose production. A comparison of basal hepatic glucose production and that measured later in the study would indicate the degree of suppression. (15,71). Insulin normally suppresses basal hepatic glucose production from 80 to 95%, and this suppression has been observed to be nearly identical in normal and uremic subjects (21,25,60). Problems with the infusate volumes and sample volumes required are in the process of being corrected in this technique, and should provide valuable information if perfected.

In the absence of direct measurements to prove that hepatic glucose production is normally suppressed in the uremic group, the numerous reports cited above which have supported the finding strongly suggest that failure of insulin to suppress hepatic glucose production is not the mechanism of insulin resistance in uremia. The possibility which then remains is that it is the resistance to insulin effect in muscle and fat which result in the observed fasting hyperinsulinemia and decreased glucose clearance under identical conditions of insulin concentration. The numerous metabolic products, ions, electrolytes and other serum components whose concentrations and metabolisms are altered in the uremic state have been discussed earlier in this work. The present study does not examine which of these might cause the alterations in glucose metabolism associated with uremia. Biochemical analyses (30,58) have pointed to inhibition of phosphofructokinase in the Embden-Meyerhof glycolytic pathway as the site at which one or more factors present in uremic subjects at levels significantly different enough from controls to inhibit this enzyme may work to produce insulin resistance.

In conclusion, study of this chronic uremic rat model by the euglycemic insulin clamp technique resulted in the findings of fasting hyperinsulinemia, fasting hyperglycemia, and a markedly diminished response to exogenous insulin resulting in diminished glucose clearance. These results support the validity of this model for the study of uremic glucose intolerance.

References:

1. Altszuler, N., Barkai, C., Bjerknes, B., Gottlieb, B., Steele, R., "Glucose Turnover Values in the Dog Obtained with Various Species of Labelled Glucose", American Journal of Physiology, 229(6):1662, 1975.
2. Amend, W.J., Steinberg, S.M., Lowrie, E.G., Lazarus, J.M., Soeldner, J.S., Hampers, C.L., Merrill, J.P., "The Influence of Serum Calcium and Parathyroid Hormone upon Glucose Metabolism in Uremia", Journal of Laboratory and Clinical Medicine, 86(3):435, 1975.
3. Bagdade, J.D., "Disorders of Glucose Metabolism in Uremia", Advances in Nephrology, Volume 8, pages 87-100, 1979.
4. Bagdade, J.D., Yee, E., Shafrir, E., "Evidence for an Accelerated Adaptation to Starvation in Chronic Uremia", Metabolism 26:1107, 1977.
5. Balestri, P.L., Rindi, P., Biagini, M., Giovanetti, S., "Effects of Uremic Serum, Urea, Creatinine, and Methylguanidine on Glucose Metabolism", Clinical Science, 42:395, 1972.
6. Boucot, N.G., Nurser, E.K., Merrill, J.B., "Carbohydrate Metabolism in Rats with Chronic Uremia", American Journal of Physiology 198:797, 1960.
7. Briggs, J.D., Buchanan, K.D., Luke, R.G., McKiddie, M.T., "Role of Insulin in Glucose Intolerance in Uremia", Lancet 1:462, 1967.
8. Cerletty, J.M., Engbring, N.H., "Azotemia and Glucose Intolerance", Annals of Internal Medicine 66(6):1097, 1967.
9. Cohen, B.D., "Guanidosuccinic Acid in Uremia", Archives of Internal Medicine 126:846, 1970.
10. Cohen, B.D., and Horowitz, H.I., "Carbohydrate Metabolism in Uremia: Inhibition of Phosphate Release," American Journal of Clinical Nutrition 21:407, 1968.
11. Crofford, O.B., and Renold, A.E., "Glucose Uptake by Incubated Rat Epididymal Adipose Tissue: Rate Limiting Steps and Site of Insulin Action", Journal of Biology and Chemistry 240:14, 1965.

12. Curry,D.L., Bennett,L.L., and Grodsky,G.M., "Dynamics of Insulin Secretion by the Perfused Rat Pancreas", Endocrinology 83:572, 1968.
13. Czech,M.P., "Insulin Action and the Regulation of Hexose Transport", Diabetes 29:399, 1980.
14. Davidson, M.B., Lowrie,E.G., and Hampers, C.L., "Lack of a Dialyzable Insulin Antagonist in Uremia", Metabolism 18(5):387, 1969.
15. De Fronzo, R.A., "Pathogenesis of Glucose Intolerance in Uremia", Metabolism, Volume 27(12), Supplement 2, pages 1866-1880, December, 1978.
16. De Fronzo,R.A. and Alvestrand, A., "Glucose Intolerance in Uremia: Site and Mechanism", American Journal of Clinical Nutrition 33:1438, July 1980.
17. De Fronzo, R.A., Andres,R., Edgar,P., and Walker,W.G. "Carbohydrate Metabolism in Uremia: A Review", Medicine Volume 52(2): 469, 1973.
18. De Fronzo,R.A., and Beckles,A.D., "Glucose Intolerance Following Chronic Metabolic Acidosis in Man", American Journal of Physiology 236:E328, 1979.
19. De Fronzo, R.A. and Felig, P., "Hepatic and Peripheral Glucose Metabolism in Diabetes", Proceedings of the 10th Congress of the International Diabetes Foundation, in Diabetes, 1979.
20. De Fronzo, R.A., Felig, P., Ferrannini, E., and Wahren J., "Effect of Graded Doses of Insulin on Splanchnic and Peripheral Potassium Metabolism in Man.", American Journal of Physiology 238:E421, 1980.
21. De Fronzo, R.A., and Ferrannini, E., "Glucose Clearance is Constant Over a Wide Range of Plasma Glucose Concentrations in Man.", (submitted for publication).
22. De Fronzo,R.A., Ferrannini,E., Hendler,R., Wahren,J. and Felig, P., "Influence of Hyperinsulinemia, Hyperglycemia, and Route of Glucose Administration on Splanchnic Glucose Exchange", Proceedings of the National Academy of Science 75:5173, 1978.

23. De Fronzo, R.A., Tobin, J.D. and Andres, R., "Glucose Clamp Technique: A Method for Quantifying Insulin Secretion and Resistance", American Journal of Physiology 237(3):E 214, 1979.
24. De Fronzo, R.A., Tobin, J., Boden, G., and Andres, R., "The Role of Growth Hormone in the Glucose Intolerance of Uremia", Acta Diabetologica Latina 16:279, 1979.
25. De Fronzo, R.A., Tobin, J.D., Rowe, J.W. and Andres, R., "Glucose Intolerance in Uremia: Quantification of Pancreatic Beta Cell Sensitivity to Glucose and Tissue Sensitivity to Insulin", Journal of Clinical Investigation 62:425, 1978.
26. De Fronzo, R.A. and Wahren, J., "Glucose Intolerance in Uremia: Primacy of Tissue Insensitivity to Insulin", American Society of Nephrology Abstract, 1979.
27. Duckworth, W.C., Heinemann, H. and Goessling, M., "Enzymatic Mechanisms for Insulin and Glucagon Degradation by the Kidney", Clinical Research 24:359A, 1976.
28. Dzurik, R. and Krajei-Lezary, B., "The Effect of Uremic Serum on Carbohydrate Metabolism in Rat Diaphragm", Experientia 23:798, 1967.
29. Dzurik, R., Niederland, T.R., and Cernacek, P., "Carbohydrate Metabolism by Rat Liver Slices Incubated in Serum Obtained from Uremic Patients", Clinical Science Volume 37:409, 1969.
30. Dzurik, R. and Valovicova, E., "Glucose Utilization in Muscle During Uremia: In Vitro Study", Clin. Chim. Acta Volume 30: 137, 1970.
31. Feldman, H.A. and Singer, I., "Endocrinology and Metabolism in Uremia and Dialysis: A Clinical Review", Medicine 54(5):345, 1974.
32. Felig, P., Wahren, J., and Hendler, R., "Influence of Oral Glucose Ingestion on Splanchnic Glucose and Gluconeogenic Substrate Metabolism in Man", Diabetes 24:468, 1975.
33. Ferrannini, E., Pilo, A., Navalesi, R., and Citti, L., "Insulin Kinetics and Glucose Induced Insulin Delivery in Chronically Dialyzed Subjects: Acute Effects of Dialysis", Journal of Clinical Endocrinology and Metabolism 49(1):15, 1979.

34. Frohlich, J., Scholmerich, J., Hoppe-Seyler, G., Maier, K.P., Talke, H., Schollmeyer, P. and Gerok, W., "The Effect of Acute Uremia on Gluconeogenesis in Isolated Perfused Rat Livers", European Journal of Clinical Investigation Volume 4:453, 1974.
35. Garber, A.J., "Skeletal Muscle Protein and Amino Acid Metabolism in Experimental Chronic Uremia in the Rat", Journal of Clinical Investigation 62:623, 1978.
36. Garber, A.J., "The Regulation of Skeletal Muscle Alanine and Glutamine Formation and Release in Experimental Chronic Uremia in the Rat", Journal of Clinical Investigation 62:633, 1978.
37. Garber, A.J., Bier, D.M., Cryer, P.E., and Pagliari, A.S., "Hypoglycemia in Compensated Chronic Renal Insufficiency: Substrate Limitation of Gluconeogenesis", Diabetes Volume 23:982, 1974.
38. Gardner, L.I., Talbot, N.B., Cook, C.D., Berman, H. and Uribe, C., "The Effect of Potassium Deficiency on Carbohydrate Metabolism", Journal of Laboratory and Clinical Medicine 35:592, 1950.
39. Ginsberg, H., Olefsky, J.M., and Reaven, G.M., "Further Evidence that Insulin Resistance Exists in Patients with Chemical Diabetes", Diabetes 23:674, 1974.
40. Glaze, R.P., Morgan, J.M., and Morgan, R.E., "Uncoupling of Oxidative Phosphorylation by Ultrafiltrates of Uremic Serum", Proceedings of the Society for Experimental Biology and Medicine 125:172, 1972.
41. Grodsky, G.M., and Bennett, L.L., "Cation Requirements for Insulin Secretion in the Isolated Perfused Pancreas", Diabetes 15:910, 1966.
42. Guder, W.G. and Wieland, O.H., "Metabolism of Isolated Kidney Tubules", European Journal of Biochemistry, Volume 31:69, 1972.
43. Hampers, C.L., Lowrie, E.G., Soeldner, J.S. and Merrill, J.P., "The Effect of Uremia Upon Glucose Metabolism", Archives of Internal Medicine 126:870, 1970.
44. Hampers, C.L., Soeldner, J.S., Doak, P.B. and Merrill, J.P., "Effect of Chronic Renal Failure and Hemodialysis on Carbohydrate Metabolism", Journal of Clinical Investigation Volume 45(11):1719, 1966.

45. Hampers, C.L., Soeldner, J.S., Gleason, R.E., Bailey, G.L., Diamond, J.H., and Merrill, J.P., "Insulin-Glucose Relationships in Uremia", American Journal of Clinical Nutrition, Volume 21:414, 1968.
46. Harter, H.R., Santiago, J.V., Rutherford, W.E., Slatopolsky, E., and Klahr, S., "The Relative Roles of Calcium, Phosphorus and Parathyroid Hormone in Glucose and Tolbutamide Mediated Insulin Release", Journal of Clinical Investigation, Volume 58:359, 1976.
47. Horl, W.H., Stepinski, J. and Heidland, A., "Carbohydrate Metabolism and Uremia: Mechanisms for Glycogenolysis and Gluconeogenesis", Klinische Wochenschrift 58:1051, 1980.
48. Horton, E.S., Johnson, C. and Lebovitz, H.E., "Carbohydrate Metabolism in Uremia", Annals of Internal Medicine, Volume 68(1):63, 1968.
49. Hutchings, R.H., Hegstrom, R.M., and Scribner, B.H., "Glucose Intolerance in Patients on Long Term Intermittent Dialysis", Annals of Internal Medicine 65(2): 275, 1966.
50. Jaspar, J.B., Mako, M.E., Kuzuya, H., Blix, P., Horwitz, D.L., and Rubenstein, A.H., "Abnormalities in Circulating Beta Cell Peptides in Chronic Renal Failure: Comparison of C-Peptide, Proinsulin, and Insulin", Journal of Laboratory and Clinical Medicine 45:441, 1977.
51. Kahn, C.R., "Insulin Resistance, Insulin Insensitivity, and Insulin Unresponsiveness: A Necessary Distinction", Metabolism 22(2):1893, 1978.
52. Kobayashi, M., and Olefsky, J.M., "Effect of Experimental Hyperinsulinemia on Insulin Binding and Glucose Transport in Isolated Rat Adipocytes", American Journal of Physiology 235:E53, 1978.
53. Lebovitz, H.E., Feinglos, M.N., Bucholtz, H.K. and Lebovitz, F.L., "Potentiation of Insulin Action: A Probable Mechanism for the Anti-Diabetic Action of Sulfonylurea Drugs", Journal of Clinical Endocrinology and Metabolism 45:601, 1977.
54. Lindall, A., Carmena, R., Cohen, S., and Comty, C., "Insulin Hypersecretion in Patients on Chronic Hemodialysis: Role of Parathyroids", Clinical Endocrinology and Metabolism, 32:653, 1971.

55. Lisch, H.J., Bolanzo, K., Patsch, J., Sailer, S., and Braunsteiner, "Interactions Between Serum Calcium Concentration and Glucose Intolerance in Normal and Azotemic Subjects", Diabetologia 9:467, 1973.
56. Lowrie, E.G., Soeldner, J.S., Hampers, C.L. and Merrill, J.P., "Glucose Metabolism and Insulin Secretion in Uremic, Pre-Diabetic and Normal Subjects", Journal of Laboratory and Clinical Medicine 76(4):603, 1970.
57. Luft, R., and Cerasi, E., "Human Growth Hormone as a Regulator of Blood Glucose Concentration and a Diabetogenic Substance", Diabetologia 4:1, 1968.
58. Luke, R.G., Dinwoodie, A.J., Linton, A.L., and Kennedy, A.C., "Fructose and Glucose Tolerance in Uremia", Journal of Laboratory and Clinical Medicine 64:731, 1964.
59. Mondon, C.E., Dolkas, C.B., and Reaven, G.M., "Effect of Acute Uremia on Insulin Removal by the Isolated Perfused Rat Liver and Muscle", Metabolism 27(2):133, 1978.
60. Mondon, C.E., Dolkas, C.B., and Reaven, G.M., "The Site of Insulin Resistance in Acute Uremia", Diabetes 7(5):571, 1978.
61. Mondon, C.E., Dolkas, C.B., and Reaven, G.M., "Mechanism of Impaired Glucose Tolerance in Acute Uremia", Diabetes Volume 26, Supplement 1, Page 375, 1977.
62. Navalesi, R., Pilo, A., Lenzi, S., and Donato, L., "Insulin Metabolism in Chronic Uremia and in the Anephric State: Effect of the Dialytic Treatment", Journal of Clinical Endocrinology and Metabolism 40:70, 1975.
63. Olefsky, J.M. and Kobayashi, M., "Ability of Circulating Insulin to Chronically Regulate the Cellular Glucose Transport System", Metabolism 27:1917, 1978.
64. Perkoff, G.T., Thomas, C.L., Newton, J.D., Sellman, J.C. and Tyler, F.H., "Mechanism of Impaired Glucose Tolerance in Uremia and Experimental Hyperazotemia", Diabetes, Volume 7:375, 1958.
65. Rabkin, R. and Kitabchi, A.E., "Factors Influencing the Handling of Insulin by the Isolated Rat Kidney", Journal of Clinical Investigation 58:169, 1978.

66. Radziuk, J., Norwich, K., and Vranic, M., "Measurement and Validation of Non-steady Turnover Rates with Application to the Insulin and Glucose Systems", Federation Proceedings 33:1855, 1974.
67. Reaven, G.M., Bernstein, R., Davis, B. and Olefsky, J.M., "Nonketotic Diabetes Mellitus: Insulin Deficiency or Insulin Resistance?", American Journal of Medicine 60:80, 1976.
68. Reaven, G.M., Weisinger, J.R. and Swenson, R.S., "Insulin and Glucose Metabolism in Renal Insufficiency", Kidney International, Volume 6, Supplement 1, page 63, 1974.
69. Rubenfeld, S., and Garber, A.J., "Abnormal Carbohydrate Metabolism in Chronic Renal Failure", Journal of Clinical Investigation 58:20, 1978.
70. Rubenstein, A.H., and Spitz, I.M., "Role of the Kidney in Insulin Metabolism and Excretion", Diabetes 17:161, 1968.
71. Sacca, L., Perez, G., Carteni, G., and Rengo, F., "Evaluation of the Role of the Sympathetic Nervous System in the Glucoregulatory Response to Insulin Induced Hypoglycemia in the Rat", Endocrinology 101(4):1016, 1977.
72. Samaan, N.A., and Freeman, R.M., "Growth Hormone Levels in Severe Renal Failure", Metabolism 19:102, 1970.
73. Sherwin, R.S., Basti, C., Finkelstein, F.O., Fisher, M., Black, H., Hendler, R., and Felig, P., "Influence of Uremia and Hemodialysis on the Turnover and Metabolic Effects of Glucagon", Journal of Clinical Investigation 57:722, 1976.
74. Snedecor, G.W. and Cochran, W.G., Statistical Methods, Ames, Iowa State University Press, Sixth Edition, 1967.
75. Snyder, D., Pulido, L.B., and Kagan, A., "Dietary Reversal of the Carbohydrate Intolerance in Uremia", Proceedings of the European Dialysis and Transplant Association, Volume 5:205, 1968.
76. Soman, V. and Felig, P., "Glucagon and Insulin Binding to Liver Membranes in a Partially Nephrectomized Uremic Rat Model", Journal of Clinical Investigation 60:224, 1977.

77. Spergel,G., Bleicher,S.J., Goldberg,M., Adosman,J. and Goldner,M.C., "The Effect of Potassium on the Impaired Glucose Tolerance in Chronic Uremia", Metabolism, Volume 16:581, 1967.
78. Spitz, I.M., Rubenstein,A.H., Bersohn,I., Abrahams,C. and Lowry,C., "Carbohydrate Metabolism in Renal Disease", Quarterly Journal of Medicine 39:201, 1970.
79. Steele,R., "Influence of Glucose Loading and of Injected Insulin on Hepatic Glucose Output", Annals of the N.Y. Academy of Science 82:420, 1959.
80. Vranic,M., "Tracer Methodology and Glucose Turnover: An Overview", Federation Proceedings 33(7):1837, 1974.
81. Westervelt,F.B., "Uremia and Insulin Response", Archives of Internal Medicine 126:865, 1970.
82. Westervelt,F.B., "Abnormal Carbohydrate Metabolism in Uremia", American Journal of Clinical Nutrition Volume 21(5):423, 1968.
83. Westervelt, F.B., "Insulin Effect in Uremia", Journal of Laboratory and Clinical Medicine 74(1):79, 1969.
84. Yalow,R.S., Goldsmith,S. and Berson,S.A., "Influence of Physiologic Fluctuations in Plasma Growth Hormone on Glucose Tolerance", Diabetes 18:402, 1969.

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